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(21) International Application Number: PCT/US89/01618 (22) International Filing Date: 20 April 1989 (20.04.89) (30) Priority data: 188,918 2 May 1988 (02.05.88) US (71) Applicant: THE UNITED STATES OF AMERICA as represented by THE SECRETARY, U.S. DEPARTMENT OF COMMERCE [US/US]; 5285 Port Royal Road, Springfield, VA 22161 (US). (72) Inventors: MILLER, Louis, H. ; 4824 Cumberland Avenue, Chevy Chase, MD 20815 (US). KASLOW, David, C. ; 4103 Garrett Park Road, Silver Spring, MD 20906 (US). (74) Agents: STERN, Marvin, R. et al.; Fleit, Jacobson, Cohn, Price, Holman & Stern, The Jenifer Building, 400 Seventh Street, N.W., Washington, DC 20004-2201 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: GENE FOR ENCODING A HUMAN MALARIA VACCINE ANTIGEN		
(57) Abstract The invention is a gene for expressing antigens for producing a human malaria vaccine. The gene includes a cloned nucleotide sequence or segment for encoding the 25kDa surface protein of zygotes and ookinetes of <i>Plasmodium falciparum</i> .		

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1 GENE FOR ENCODING A HUMAN
2 MALARIA VACCINE ANTIGEN

3 BACKGROUND OF THE INVENTION

4 1. Field of the Invention

5 This invention relates to genes for encoding malarial
6 vaccine antigens. More specifically, this invention
7 relates to a gene for encoding the Plasmodium falciparum 25
8 kDA ookinete surface antigen.

9 2. Description of the Background Art

10 Malaria continues to exact a heavy toll from mankind.
11 Approximately 25 percent of all deaths of children in rural
12 Africa between the ages of one to four years are caused by
13 Malaria. This death rate continues despite the sensitivity
14 of local parasites to chloroquine. Mosquito control is
15 difficult in this setting. The greatest hope at present
16 for reducing this mortality rate is a protective vaccine
17 that reduces the incidence of the disease and death by
18 suppressing the replication of the parasite. The major
19 cause of malaria in humans is the parasite, Plasmodium
20 falciparum.

1 The value of various vaccines to combat malaria is
2 appreciated through an understanding of the life cycle of
3 the parasite. Infection in man begins when young malarial
4 parasites or "sporozoites" are injected into the blood
5 stream of a human by the mosquito. After injection the
6 parasite localizes in liver cells. After approximately one
7 week the parasites or "merozoites" are released into the
8 blood stream. The entry of the parasites into the blood
9 stream begins the "erythrocytic" phase. Each parasite
10 enters a red blood cell in order to grow and develop. When
11 the merozoite matures in the red blood cell, it is known as
12 a trophozoite and schizont. A schizont is the stage when
13 nuclear division occurs to form individual merozoites which
14 are released to invade other red cells. After several
15 schizogonic cycles, some parasites, instead of becoming
16 schizonts through asexual reproduction, develop into large
17 uninucleate parasites. These parasites undergo sexual
18 development.

19 Sexual development of the malaria parasites involves
20 the female or "macrogametocyte" and the male parasite or
21 "microgametocyte". These gametocytes do not undergo any
22 further development in man. Upon ingestion of the
23 gametocytes into the mosquito, the complicated sexual cycle
24 begins in the midgut of the mosquito. The red blood cells
25 disintegrate in the midgut of the mosquito after 10 to 20
26 minutes. The microgametocyte continues to develop through
27 exflagellation and releases 8 highly flagellated
28 microgametes. Fertilization occurs with the fusion of the
29 microgamete into a macrogamete. The fertilized parasite is
30 known as a zygote that develops into an "ookinete". The
31 ookinete penetrates the midgut wall of the mosquito and
32 transforms into the oocyst within which many small
33 sporozoites form. When the oocyst ruptures the sporozoites
34 migrate to the salivary gland of the mosquito via the

1 haemolymph. Once in the saliva of the mosquito, the
2 parasite can be injected into a host.

3 Malaria vaccines are being developed against different
4 stages in the parasite's life-cycle which includes the
5 sporozoite, asexual erythrocyte, and sexual stage. Each
6 development increases the opportunity to control malaria in
7 the many diverse settings within which the disease occurs.
8 Sporozoite vaccines would prevent mosquito-induced
9 infections. First generation vaccines of this type have
10 been tested in humans. Asexual erythrocytic stage vaccines
11 would be useful in reducing the severity of the disease.
12 Multiple candidate antigens have been cloned and tested in
13 animals and in humans.

14 Sexual stage vaccines would induce antibodies which,
15 when ingested in a bloodmeal containing sexual stage
16 parasites, would prevent infection of mosquitoes. Although
17 not directly protective against infection or disease, the
18 sexual stage vaccine combined with a protective vaccine
19 such as a sporozoite or asexual stage vaccine would reduce
20 the chance of transmission of vaccine-induced mutants
21 resistant to the protective component. In this manner, the
22 useful life of the protective component would be
23 lengthened. In some geographical areas the sexual stage
24 vaccine could reduce transmission below the critical
25 threshold required to maintain the infected population.
26 This reduced transmission would be useful in assisting in
27 the control or eradication of malaria.

28 U.S. Patent Number 4,632,909 to Carter and Miller,
29 herein incorporated by reference, discloses monoclonal
30 antibodies that bind with one or more proteins located on
31 the surface of gametes or zygotes of malaria parasites and

1 are targets for sexual stage vaccines. These antibodies
2 are specific for antigens on mosquito midgut stages of
3 malaria parasite and sterilize the parasites in mosquitoes
4 otherwise capable of transmitting the disease. The
5 monoclonal antibodies are specific for the 255, 59 and 53 K
6 surface proteins on Plasmodium falciparum and for the 25 K
7 surface protein on zygotes and ookinetes of Plasmodium
8 gallinaceum. This invention includes a process for
9 blocking transmissions of malaria parasites. The process
10 involves the feeding of mosquitoes carrying the malaria
11 parasite monoclonal antibodies specific for a glycoprotein
12 on the surface of the malaria parasite zygote. The
13 glycoprotein has a molecular weight of 24-30 K. The
14 process is effective in a zygote up to about 3 hours after
15 fertilization. This invention does not involve a cloned
16 gene to induce transmission blocking immunity to malaria
17 nor a deduced peptide from the gene for use in a malarial
18 vaccine.

19 A study to identify antigens useful to develop malaria
20 transmission blocking immunity is disclosed in the article,
21 Carter et al., "Target Antigens in Malaria Transmission
22 Blocking Immunity," Phil. Trans. R. Soc. Land. B
23 307:201-213 (1984), herein incorporated by reference. This
24 article describes the phases of development of malaria
25 parasites wherein transmission blocking immunity occurs.
26 Target antigens on gametes and newly fertilized zygotes and
27 target antigens of post-fertilization transmission blocking
28 immunity are identified in the article. This article does
29 not disclose a cloned gene to induce transmission blocking
30 immunity to malaria nor a deduced peptide from the gene for
31 use in a malarial vaccine.

32 An article, Grotendorst et al., "A Surface Protein
33 Expressed During the Transformation of Zygotes of

1 Plasmodium gallinaceum is a Target of Transmission-Blocking
2 Antibodies," Infection and Immunity, Vol. 45, No. 3, p.
3 775-777 (1984), herein incorporated by reference, discloses
4 a specific protein suitable for use as an antigen. This
5 article identifies materials and procedures that are useful
6 in isolating and identifying an antigen which is a surface
7 protein of M_r 26,000 synthesized by zygotes of P.
8 gallinaceum. Monoclonal antibodies, having properties of
9 anti-ookinete serum, were found in certain examples to
10 suppress infectivity of fertilized parasites to
11 mosquitoes. An analogous 25kDa surface protein synthesized
12 by zygotes and ookinetes of Plasmodium falciparum is
13 described by Vermeulen et al., "Sequential Expression of
14 Antigens on Sexual Stages of Plasmodium Falciparum
15 accessible to Transmission-blocking Antibodies in the
16 Mosquito," J. Exp. Med. 162:1460-1476 (1985), herein
17 incorporated by reference. These articles do not disclose
18 a cloned gene of P. falciparum to induce transmission
19 blocking immunity to malaria nor a deduced peptide from the
20 gene for use in a malarial vaccine.

21 The industry is lacking a gene which can produce a
22 vaccine designed to induce transmission blocking immunity
23 to Plasmodium falciparum 25kDa surface protein (herein
24 after Pfs25) and other sexual stage antigens. The industry
25 is also lacking a synthetic peptide that can be expressed
26 from the above gene and used in a pharmaceutical
27 composition to produce a malarial vaccine. Vaccines derived
28 from such genes would prolong the usefulness of other
29 protective malarial vaccines as well as reduce the spread
30 of malaria in areas of low transmission.

1

SUMMARY OF THE INVENTION

22 The invention is a gene for expressing antigens for
33 producing a human malaria vaccine. The gene includes a
4 cloned nucleotide sequence or segment for encoding the
5 25kDa surface protein of zygotes and ookinetes of
6 Plasmodium falciparum. The segment of the gene encoding
7 the protein is

8. ATG AAT AAA CTT TAC AGT TTG TTT CTT TTC CTT TTC ATT CAA CTT
9. AGC ATA AAA TAT AAT AAT GCG AAA GTT ACC GTG GAT ACT GTA TGC
100 AAA AGA GGA TTT TTA ATT CAG ATG AGT GGT CAT TTG GAA TGT AAA
11. TGT GAA AAT GAT TTG GTG TTA GTA AAT GAA GAA ACA TGT GAA GAA
12. AAA GTT CTG AAA TGT GAC GAA AAG ACT GTA AAT AAA CCA TGT GGA
13 GAT TTT TCC AAA TGT ATT AAA ATA GAT GGA AAT CCC GTT TCA TAC
14 GCT TGT AAA TGT AAT CTT GGA TAT GAT ATG GTA AAT AAT GTT TGT
15 ATA CCA AAT GAA TGT AAG AAT GTA ACT TGT GGT AAC GGT AAA TGT
16. ATA TTA GAT ACA AGC AAT CCT GTT AAA ACT GGA GTT TGC TCA TGT
17. AAT ATA GGC AAA GTT CCC AAT GTA CAA GAT CAA AAT AAA TGT TCA
18 AAA GAT GGA GAA ACC AAA TGC TCA TTA AAA TGC TTA AAA GAA AAT
19 GAA ACC TGT AAA GCT GTT GAT GGA ATT TAT AAA TGT GAT TGT AAA
20 GAT GGA TTT ATA ATA GAT AAT GAA AGC TCT ATA TGT ACT GCT TTT
21 TCA GCA TAT AAT ATT TTA AAT CTA AGC ATT ATG TTT ATA CTA TTT
22 TCA GTA TGC TTT TTT ATA ATG TAA.

23 The invention includes a synthetic protein which is
24 useful for preparing a malaria vaccine. The synthetic
25 protein of the cloned gene is

26 Met Asn Lys Leu Tyr Ser Leu Phe Leu Phe Leu Phe Ile Gln Leu
27 Ser Ile Lys Tyr Asn Asn Ala Lys Val Thr Val Asp Thr Val Cys
28 Lys Arg Gly Phe Leu Ile Gln Met Ser Gly His Leu Glu Cys Lys
29 Cys Glu Asn Asp Leu Val Leu Val Asn Glu Glu Thr Cys Glu Glu

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1 Lys Val Leu Lys Cys Asp Glu Lys Thr Val Asn Lys Pro Cys Gly
2 Asp Phe Ser Lys Cys Ile Lys Ile Asp Gly Asn Pro Val Ser Tyr
3 Ala Cys Lys Cys Asn Leu Gly Tyr Asp Met Val Asn Asn Val Cys
4 Ile Pro Asn Glu Cys Lys Asn Val Thr Cys Gly Asn Gly Lys Cys
5 Ile Leu Asp Thr Ser Asn Pro Val Lys Thr Gly Val Cys Ser Cys
6 Asn Ile Gly Lys Val Pro Asn Val Gln Asp Gln Asn Lys Cys Ser
7 Lys Asp Gly Glu Thr Lys Cys Ser Leu Lys Cys Leu Lys Glu Asn
8 Glu Thr Cys Lys Ala Val Asp Gly Ile Tyr Lys Cys Asp Cys Lys
9 Asp Gly Phe Ile Ile Asp Asn Glu Ser Ser Ile Cys Thr Ala Phe
10 Ser Ala Tyr Asn Ile Leu Asn Leu Ser Ile Met Phe Ile Leu Phe
11 Ser Val Cys Phe Phe Ile Met.

12 The invention includes a pharmaceutical composition
13 having the synthetic protein and the method to make an
14 anti-malarial vaccine including the synthetic protein.

15 BRIEF DESCRIPTION OF THE DRAWINGS

16 Figure 1 illustrates the neucleotide and predicted
17 amino acid sequence of Pfs25.

18 Figure 2 illustrates a Northern blot analysis of
19 asexual and sexual stage RNA.

20 Figure 3 illustrates a protein structure of Pfs25
21 arranged to emphasize the relatedness of the EGF-like
22 domains.

23 DETAILED DESCRIPTION OF THE INVENTION

24 The invention is the isolated and cloned gene for
25 encoding the 25 kDa surface protein (Pfs25) of zygotes and
26 ookinetes of Plasmodium falciparum. The deduced amino acid
27 sequence of this gene consists of a signal sequence, a
28 hydrophobic C-terminus, and four tandem epidermal growth

1 factor (EGF)-like domains. The cloned gene of this
2 invention, therefore, provides a useful composition that
3 can express an antigen that is useful in preparing a
4 malarial vaccine. The antigen is also a useful product of
5 this invention. The antigen is a polypeptide that can be
6 used in therapeutic quantities to prepare pharmaceutical
7 compositions which are suitable as malaria vaccines.

8 The genes for encoding the three sexual stage-specific
9 antigens of P. falciparum have not been cloned to date.
10 This is in part due to the fact that monoclonal antibody
11 defined epitopes are dependent on disulfide bonds and large
12 quantities of parasites and purified protein needed for
13 peptide sequencing are difficult to obtain. The gene of
14 this invention is obtained by purifying Pfs25 from zygotes
15 of P. falciparum. In the preferred embodiment of the
16 invention the 3D7 clone of NF54 P. falciparum is used. The
17 Pfs25 is purified by using immunoaffinity chromatography
18 and SDS-PAGE. The microsequence of the protein is then
19 performed in order to make oligonucleotide probes to screen
20 genomic DNA libraries. The purified Pfs25 is digested
21 with trypsin, because the amino-terminus was blocked. The
22 resulting peptides are fractionated by HPLC. Five peptide
23 sequences are obtained from this process. These peptide
24 sequences are identified in Figure 1.

25 A highly degenerate oligonucleotide probe is
26 constructed from tryptic peptide sequence. This
27 oligonucleotide is used to clone a 600 bp Dra I fragment
28 (pSKR 2) of genomic DNA. The Dra I fragment contains one
29 long open reading frame but no termination codon.
30 Therefore, a 3.5 kb Hind III fragment (pNF4.13) is cloned.
31 Both cloned fragments can be used to determine the
32 nucleotide sequence of Pfs25.

Figure 1 illustrates the amino acid sequence for Pfs25 as deduced by the above process. This amino acid sequence is shown above the nucleotide sequence. The gene has a single exon that codes for a polypeptide of 217 amino acids. The predicted coding region of the nucleotide sequence is capitalized and consists of 654 base pairs. The N-terminus is blocked; therefore, the start of the mature protein has not been determined. Solid lines represent tryptic peptide sequences determined by microsequencing. Asterisks represent the sequence determined by microsequencing radiolabelled peptides. The dotted line represents the indeterminate amino acid residue of the microsequenced peptide. The double solid line represents the tryptic peptide sequence used to construct oligonucleotide probes. The open circles represent the sites of possible asparagine-linked glycosylation. The broken lines represent the hydrophobic regions. First and most important in providing evidence that the gene for the the 25kDa ookinete surface antigen is cloned is that five of the microsequenced tryptic peptides of Pfs25 are found within the deduced amino acid sequence of Pfs25 as shown in Figure 1. A preparation of (³⁵S)-labelled Pfs25 can be immunopurified from zygotes that had been metabolically labelled with ³⁵S-methionine and ³⁵S-cysteine. The resulting tryptic peptides of Pfs25 are separated by HPLC and the fraction containing the most radioactivity is microsequenced. The radioactive peaks for cysteine and methionine in the tryptic peptide of Pfs25 perfectly match the position of these residues in the deduced amino acid sequence of the Pfs25 gene of Figure 1 marked by asterisks.

A malarial vaccine can be produced from the gene of Figure 1 or a portion thereof. The gene can be modified using known techniques to delete the signal sequence, the

1 hydrophobic anchor, and/or other portions of the gene.
2 These modifications to the gene continue to produce a
33 suitable antigen for use in the vaccine.

4 Those skilled in the art can understand that certain
5 codons presented in the sequence of Figure 1 can be
6 substituted by other codons and produce an equivalent
7 segment. The polypeptide produced by the equivalent
8 segment has a corresponding change in its amino acid
9 sequence, but has an equivalent function to the preferred
10 polypeptide. For example, a substitution of glutamic acid
11 for ~~glycine~~ at amino acid 131 does not alter the function
12 of the polypeptide.

13 Second, the gene of this invention is expressed
14 preferentially in the sexual stages of P. falciparum. This
15 is the stage in which Pfs25 is synthesized. The mRNA from
16 gametocytes (not shown) and five hour old zygotes as
17 illustrated in Figure 2, lane 5, of P. falciparum using the
18 3D7 clone of NF 54, have an abundance of an approximately
19 1.4 kb species that hybridizes to pSKR 2. In contrast, the
20 mRNA from asexual stage parasites of 3D7 as illustrated in
21 Figure 2, lane A, gives only a weak signal. The weak
22 signal from the asexual stage parasites is most likely due
23 to the presence of some gametocytes in the preparation of
24 parasites. For example pSKR 2 does not hybridize with the
25 mRNA as illustrated in Figure 2, lane L, from a P.
26 falciparum parasite that produces no gametocytes such as
27 the LF4 clone of a Liberian isolate of P. falciparum.

28 Third, when gametes, zygotes, or ookinetes of P.
29 falciparum are metabolically labeled, multiple sexual stage
30 proteins incorporate ³⁵S-methionine and Pfs25 is not a
31 major radiolabelled product. In contrast, Pfs25 is the
32 predominate product incorporating ³⁵S-cysteine as known

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1 by tests which are standard in the art. The deduced amino
2 acid sequence contains 11 percent cysteine which correlates
3 with these results.

4 Fourth, the structure of the protein from the deduced
5 amino acid sequence is consistent with previous reports as
6 cited above of the biochemistry of the 25 kDa surface
7 glycoprotein. The deduced sequence contains a putative
8 signal peptide at the N-terminus and a short hydrophobic
9 anchor at the C-terminus. It has four potential
10 glycosylation sites for N-linked sugars and encodes for a
11 polypeptide of approximately 24 kDa. The deduced sequence
12 also has a short hydrophobic anchor of 15 amino acids and
13 the lack of a potential cytoplasmic hydrophilic region at
14 the C-terminus.

15 The organization of the cysteines between the signal
16 sequence and the hydrophobic C-terminus is similar to the
17 domains in EGF 1 as illustrated in Figure 3B. Based on the
18 position of the cysteines in the exons of human EGF
19 precursor and human LDL receptor, three cysteine residues
20 precede the consensus sequence Y/F-x-C x-C x-x-G-Y/F and
21 one follows it as illustrated in Figure 3B. The EGF-like
22 domain is also found in invertebrates such as notch in
23 Drosophila melanogaster and lin-12 in Caenorhabditis
24 elegans. This invention provides the first report of the
25 presence of EGF-like domains in proteins of unicellular
26 organisms. The presence of EGF-like domains in the protein
27 of this invention can be expected to have a growth factor
28 effect on higher organisms including the mosquito.

29 EXAMPLE

30 The following example provides the procedure for
31 obtaining the gene of the invention. This example

1 represents the preferred embodiment of the invention.

22 A 3D7 clone of an NF 54 isolate of P. falciparum was
33 cultured in vitro and zygotes were prepared as described
4 above. Pfs25 from Triton X-100 extracts of five hour old
5 zygotes (10^9) was immunoaffinity purified using monoclonal
6 antibody 1C7 covalently linked to Sepharose 4B beads
7 (CnBr-activated). Pfs25 had been metabolically labeled
8 with trans S35 which has 70% ^{35}S -methionine, 20%
9 ^{35}S -cysteine, and 10% other ^{35}S -compounds and is
10 commercially available from ICN Radiochemicals, Inc. The
11 beads contained Pfs25 and were resuspended in SDS-PAGE
12 sample buffer having 5% SDS, 62.5mM Tris at pH 6.8, 0.002%
13 Bromophenol blue, and 8 M urea. These were heated at 68°C
14 for five minutes. The eluted protein in the sample buffer
15 was loaded onto a 12% SDS-polyacrylamide gel under
16 nonreducing conditions. The Pfs25 was the only
17 radiolabelled protein identified on the gel and was
18 recovered from the gel by passive diffusion. The
19 lyophilized sample was resolubilized in 0.5 M Tris-HCl pH
20 8.5, 6 M guanidine hydrochloride, 0.3 mM EDTA buffer
21 containing 64 mM dithiothreitol, and incubated for 2 hours
22 at 37°C in a N_2 atmosphere. Iodoacetamide was added to a
23 final concentration of 174 mM and reacted for 1 hour at
24 25°C in the dark. An excess of 2-mercaptoethanol was added
25 followed by 10 volumes of absolute ethanol. The reduced
26 and alkylated protein was allowed to precipitate at -20°C
27 for 4 hours. The remaining pellet was resuspended in 50 mM
28 NH_4HCO_3 , pH 7.9 and digested with two 0.5 ug doses of TPCK
29 treated trypsin (Sigma), each dose being followed by a 6
30 hour incubation at 37°C. The digestion was terminated by
31 heating the sample for 10 minutes at 65°C. The tryptic
32 peptides were fractionated on a reverse phase HPLC (RP-300,
33 Applied Biosystems, Inc.) using an 50% acetonitrile

1 gradient with 0.1% trifluoroacetic acid. Peptide
2 microsequencing was performed on a model 470A gas phase
3 sequencer from Applied Biosystems, Inc. 40% of each cycle's
4 product was analyzed on an attached model 120A PTH analyzer
5 using the manufacturer's program, C3RPTH. For one peptide,
6 the radioactivity of the remaining 60% of each cycle's
7 product was determined. Radioactive peaks (***) were found
8 in cycle numbers 1, 7, 12, and 17.

9 The peptide sequence identified by double solid lines
10 was used to construct highly degenerate oligonucleotide
11 probes. A group of oligonucleotides of 512 degeneracy
12 hybridized to a 1.4 kb band of zygote RNA. By varying the
13 codon for proline, the probe was divided into four groups
14 of 128 degeneracy each, one group of which hybridized to a
15 1.4 kb band of RNA as well as a 600 bp Dra I fragment of
16 genomic DNA. 20,000 plaques, of a size-selected Dra I
17 fragment library in lambda gt 10, were screened with this
18 oligonucleotide probe. One clone was identified and
19 subcloned into Blue-script SK (pSKR 2). pSKR 2 was used to
20 identify a 3.5 kb Hind III fragment in a size selected
21 library in the vector pSP64. Both strands of each clone
22 were sequenced by the dideoxynucleotide terminator method.

23 In Figure 2 the Northern blot analysis of asexual and
24 sexual stage RNA is shown. Total cellular RNA (20 ug/lane)
25 was prepared from asexual parasites (L) of LF 4, asexual
26 parasites (A) of 3D7, or 5 hour old zygotes (S) of 3D7 were
27 electrophoresed through a 1% agarose/formaldehyde gel and
28 transferred to a nylon membrane. The filter was hybridized
29 at 55°C overnight with randomly primed pSKR 2 insert
30 (specific activity 10^9 c.p.m. μg^{-1}) and washed as described
31 Size markers are 0.24-9.5 kb RNA ladder (BRL).

32 Figure 3 illustrates in part A protein structure of
33 Pfs25 arranged to emphasize the relatedness of the EGF-like

1 domains. The boxes represent cysteine residues and other
2 identical or related amino acids. Part B illustrates
3 EGF-like repeat consensus sequence from Pfs25, lin-12,
4 notch, EGF, and human LDL receptor. The boxes represent
5 cysteine residues. The double box represents a core of
6 consensus sequence. The letters designate the following
7 amino acids: C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; I,
8 Ile; K; Lys; L, Leu; N, Asn; T, Thr; Y, Tyr; x, any amino
9 acid; and unspecified number of amino acids.

IN THE CLAIMS

1. A gene for expressing antigens for producing a human malaria vaccine consisting essentially of a cloned gene for encoding the 25 kDa surface protein or a portion thereof of zygotes and ookinetes of Plasmodium falciparum.

2. The coding region of said gene of claim 1 wherein said nucleotide segment is

ATG AAT AAA CTT TAC AGT TTG TTT CTT TTC CTT TTC ATT CAA CTT
AGC ATA AAA TAT AAT AAT GCG AAA GTT ACC GTG GAT ACT GTA TGC
AAA AGA GGA TTT TTA ATT CAG ATG AGT GGT CAT TTG GAA TGT AAA
TGT GAA AAT GAT TTG GTG TTA GTA AAT GAA GAA ACA TGT GAA GAA
AAA GTT CTG AAA TGT GAC GAA AAG ACT GTA AAT AAA CCA TGT GGA
GAT TTT TCC AAA TGT ATT AAA ATA GAT GGA AAT CCC GTT TCA TAC
GCT TGT AAA TGT AAT CTT GGA TAT GAT ATG GTA AAT AAT GTT TGT
ATA CCA AAT GAA TGT AAG AAT GTA ACT TGT GGT AAC GGT AAA TGT
ATA TTA GAT ACA AGC AAT CCT GTT AAA ACT GGA GTT TGC TCA TGT
AAT ATA GGC AAA GTT CCC AAT GTA CAA GAT CAA AAT AAA TGT TCA
AAA GAT GGA GAA ACC AAA TGC TCA TTA AAA TGC TTA AAA GAA AAT
GAA ACC TGT AAA GCT GTT GAT GGA ATT TAT AAA TGT GAT TGT AAA
GAT GGA TTT ATA ATA GAT AAT GAA AGC TCT ATA TGT ACT GCT TTT
TCA GCA TAT AAT ATT TTA AAT CTA AGC ATT ATG TTT ATA CTA TTT
TCA GTA TGC TTT TTT ATA ATG TAA.

3. The gene of claim 2 wherein said nucleotide segment encodes said 25kDa surface protein of zygotes and ookinetes, said surface protein having a signal sequence, a hydrophobic C-terminus, and four tandem epidermal growth factor-like domains.

4. A malarial vaccine comprising said surface protein of claim 3.

1 5. A synthetic peptide for producing a human malaria
2 vaccine consisting essentially of the 25 kDa surface
3 protein or a portion thereof of zygotes and ookinetes of
4 Plasmodium falciparum produced by a cloned gene.

5 6. The gene of claim 5 wherein said surface protein
6 has a deduced amino acid segment is

7 Met Asn Lys Leu Tyr Ser Leu Phe Leu Phe Leu Phe Ile Gln Leu
8 Ser Ile Lys Tyr Asn Asn Ala Lys Val Thr Val Asp Thr Val Cys
9 Lys Arg Gly Phe Leu Ile Gln Met Ser Gly His Leu Glu Cys Lys
10 Cys Glu Asn Asp Leu Val Leu Val Asn Glu Glu Thr Cys Glu Glu
11 Lys Val ~~Leu~~ Lys Cys Asp Glu Lys Thr Val Asn Lys Pro Cys Gly
12 Asp Phe ~~Ser~~ Lys Cys Ile Lys Ile Asp Gly Asn Pro Val Ser Tyr
13 Ala Cys Lys Cys Asn Leu Gly Tyr Asp Met Val Asn Asn Val Cys
14 Ile Pro Asn Glu Cys Lys Asn Val Thr Cys Gly Asn Gly Lys Cys
15 Ile Leu Asp Thr Ser Asn Pro Val Lys Thr Gly Val Cys Ser Cys
16 Asn Ile Gly Lys Val Pro Asn Val Gln Asp Gln Asn Lys Cys Ser
17 Lys Asp Gly Glu Thr Lys Cys Ser Leu Lys Cys Leu Lys Glu Asn
18 Glu Thr Cys Lys Ala Val Asp Gly Ile Tyr Lys Cys Asp Cys Lys
19 Asp Gly Phe Ile Ile Asp Asn Glu Ser Ser Ile Cys Thr Ala Phe
20 Ser Ala Tyr Asn Ile Leu Asn Leu Ser Ile Met Phe Ile Leu Phe
21 Ser Val Cys Phe Phe Ile Met.

22 7. A malarial vaccine comprising said surface protein
23 of claim 6.

24 8. A pharmaceutical composition comprising the 25kDa
25 surface protein or portion thereof of zygotes and ookinetes
26 of Plasmodium falciparum produced by a cloned gene, said
27 protein being in a therapeutically effective concentration
28 to provide a growth factor effect on higher organisms.

1 / 3

aattgttgtaaaaagaaaaaacaaaaaaaaaaaaaaaaaactcataccttatatttttttattcttttaaaa

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1  Met Asn Lys Leu Tyr Ser Leu Phe Leu Phe Leu Phe Ile Gln Leu Ser Ile Lys Tyr
1  ATG AAT AAA CTT TAC AGT TTG TTT CTT TTC CTT TTC ATT CAA CTT AGC ATA AAA TAT

20  Asn Asn Ala Lys Val Thr Val Asp Thr Val Cys Lys Arg Gly Phe Leu Ile Gln Met
58  AAT AAT GCG AAA GTT ACC GTG GAT ACT GTA TGC AAA AGA GGA TTT TTA ATT CAG ATG

39  Ser Gly His Leu Glu Cys Lys Cys Glu Asn Asp Leu Val Leu Val Asn Glu Glu Thr
115 AGT GGT CAT TTG GAA TGT AAA TGT GAA AAT GAT TTG GTG TTA GTA AAT GAA GAA ACA

58  Cys Glu Glu Lys Val Leu Lys Cys Asp Glu Lys Thr Val Asn Lys Pro Cys Gly Asp
172 TGT GAA GAA AAA GTT CTG AAA TGT GAC GAA AAG ACT GTA AAT AAA CCA TGT GGA GAT

77  Phe Ser Lys Cys Ile Lys Ile Asp Gly Asn Pro Val Ser Tyr Ala Cys Lys Cys Asn
229 TTT TCC AAA TGT ATT AAA ATA GAT GGA AAT CCC GTT TCA TAC GCT TGT AAA TGT AAT

96  Leu Gly Tyr Asp Met Val Asn Asn Val Cys Ile Pro Asn Glu Cys Lys Asn Val Thr
286 CTT GGA TAT GAT ATG GTA AAT AAT GTT TGT ATA CCA AAT GAA TGT AAG AAT GTA ACT

115 Cys Gly Asn Gly Lys Cys Ile Leu Asp Thr Ser Asn Pro Val Lys Thr Gly Val Cys
343 TGT GGT AAC GGT AAA TGT ATA TTA GAT ACA AGC AAT CCT GTT AAA ACT GGA GTT TGC

134 Ser Cys Asn Ile Gly Lys Val Pro Asn Val Gln Asp Gln Asn Lys Cys Ser Lys Asp
400 TCA TGT AAT ATA GGC AAA GTT CCC AAT GTA CAA GAT CAA AAT AAA TGT TCA AAA GAT

153 Gly Glu Thr Lys Cys Ser Leu Lys Cys Leu Lys Glu Asn Glu Thr Cys Lys Ala Val
457 GGA GAA ACC AAA TGC TCA TTA AAA TGC TTA AAA GAA AAT GAA ACC TGT AAA GCT GTT

172 Asp Gly Ile Tyr Lys Cys Asp Cys Lys Asp Gly Phe Ile Ile Asp Asn Glu Ser Ser
514 GAT GGA ATT TAT AAA TGT GAT TGT AAA GAT GGA TTT ATA ATA GAT AAT GAA AGC TCT

191 Ile Cys Thr Ala Phe Ser Ala Tyr Asn Ile Leu Asn Leu Ser Ile Met Phe Ile Leu
571 ATA TGT ACT GCT TTT TCA GCA TAT AAT ATT TTA AAT CTA AGC ATT ATG TTT ATA CTA

210 Phe Ser Val Cys Phe Phe Ile Met
628 TTT TCA GTA TGC TTT TTT ATA ATG TAA atattataacaacatatatatatttttaaatggtaaa

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FIG. 1

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FIG. 2

L A S
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-9.5
-7.5

-4.4

-2.4

-1.4

-.24

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FIG. 3A

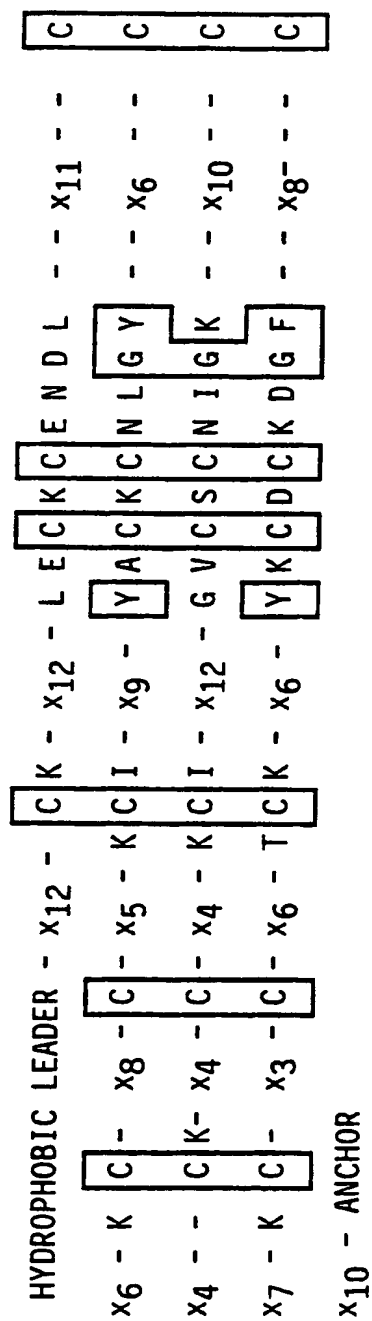
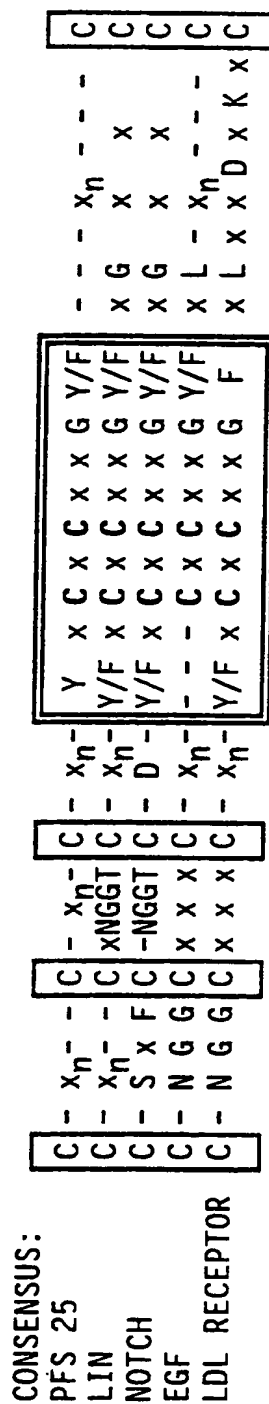


FIG. 3B



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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/01618

I. CLASSIFICATION OF SUBJECT MATTER ⁶ (If several classification symbols apply, indicate all)		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4): CO7K 7/10; A61K 37/02; CO7H 15/12 U.S. Cl: 530/350,324; 536/27; 424/88; 514/12		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	530/350,324; 514/12; 424/88; 536/27	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US, A, 4,632,909 (Carter), 30 December 1986 See the entire document.	5-8
X	J. Exp. Med., vol. 162, issued November 1985 VERMEULEN, "Sequential expression of anti- gens on sexual stages of plasmodium falciparum accessible to transmission-blocking antibodies in the mosquito", pages 1460-1476 See the entire article.	5,6
X,P	CHEMICAL ABSTRACTS, Volume 109, No. 19, issued 1988 (Columbus, Ohio, USA), Kaslow, "A vaccine candidate from the sexual stage of human malaria that contains EGF-like domains". See page 547, column 2, abstract no. 168474a, Nature (London), 1988, 333(6168), 74-6 (Eng).	1-8
A	US, A, 4,707,357, (DAME), 17 November 1987, See the entire document.	1-8
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
09 August 1989	04 OCT 1989	
International Searching Authority	Signature of Authorized Officer	
ISA/US	T. D. Wessendorf	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y,P	Chemical Abstracts, Volume 110, No. 9, issued 1989 (Columbus, Ohio, USA), Carter "Restricted or absent immune responses in human populations to Plasmodium Falciparum gamete antigens that are targets of malaria transmission-blocking antibodies", see page 468, col. 2 abstract no. 73510 r, J. Exp. Med. 1989, 169(1), 135-47 (Eng).	5-8
Y,P	Chemical Abstracts, Volume 110, No. 7, issued 1989 (Columbus, Ohio, USA), Holder, "Immunization against Plasmodium Falciparum with recombinant polypeptides produced in Escherichia coli", See page 502, col. 2, abstract no. 55534X, Parasite Immunol 1988, 10(6), 607-17 (Eng).	1-8
A	J. Exp Med., vol. 158, issued September 1983, RENER, "Target antigens of Transmission-blocking immunity on gametes of plasmodium falciparum", pages 976-981, See the entire article	1-8
A	The Journal of Immunology, Vol. 137, No. 3 issued 01 August 1986, CHIZZOLINI, "Antigen-Specific and MHC-restricted Plasmodium falciparum-induced human T lymphocyte clones", page 1022-1028, See the entire article.	1-8

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y

WO/A WO 87/03882 (THE WALTER AND ELIZA HALL
INSTITUTE OF MEDICAL RESEARCH), 2 July 1987.
See entire document.

1

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹³, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.